

What is claimed is:

- 1 1. A radiolabeled immunotoxin comprising a toxic domain,
2 a targeting domain, and at least one radionuclide atom,
3 wherein the targeting domain is a single-chain Fv (sFv)
4 antibody fragment that binds to a target molecule on a target
5 cell, wherein the target molecule is not an ε chain of a T
6 cell CD3 complex.
- 1 2. The radiolabeled immunotoxin of claim 1, wherein the
2 toxic domain is a toxic polypeptide selected from the group
3 consisting of: (a) ricin, (b) *Pseudomonas* exotoxin (PE); (c)
4 bryodin; (d) gelonin; (e) α-sarcin; (f) aspergillin; (g)
5 restrictocin; (h) angiogenin; (i) saporin; (j) abrin;
6 (k) pokeweed antiviral protein (PAP); (l) a ribonuclease; (m) a
7 pro-apoptotic polypeptide; and (n) a functional fragment of
8 any of (a)-(m).
- 1 3. The radiolabeled immunotoxin of claim 1, wherein the
2 toxic domain is diphtheria toxin (DT) or a functional fragment
3 thereof.
- 1 4. The radiolabeled immunotoxin of claim 3, wherein the
2 toxic domain comprises amino acids 1-389 of DT.
- 2 5. The radiolabeled immunotoxin of claim 1, wherein the
3 target cell is a cancer cell.
- 1 6. The radiolabeled immunotoxin of claim 5, wherein the
2 cancer cell is selected from the group consisting of a neural
3 tissue cancer cell, a melanoma cell, a breast cancer cell, a

4 lung cancer cell, a gastrointestinal cancer cell, an ovarian
5 cancer cell, a testicular cancer cell, a lung cancer cell, a
6 prostate cancer cell, a cervical cancer cell, a bladder cancer
7 cell, a vaginal cancer cell, a liver cancer cell, a renal
8 cancer cell, a bone cancer cell, and a vascular tissue cancer
9 cell.

1 7. The radiolabeled immunotoxin of claim 5, wherein the
2 target molecule is Her-2/neu.

1 8. The radiolabeled immunotoxin of claim 5, wherein the
2 target molecule is selected from the group consisting of a
3 mucin molecule, carcinoembryonic antigen (CEA), prostate-
4 specific antigen (PSA), folate binding receptor, A33 alpha
5 fetoprotein, CA-125 glycoprotein, colon-specific antigen p,
6 ferritin, p-glycoprotein, G250, OA3, PEM glycoprotein, L6
7 antigen, 19-9, P97, placental alkaline phosphatase, 7E11-C5,
8 17-1A, TAG-72, 40 kDa glycoprotein, URO-8, a tyrosinase, an
9 interleukin- (IL-)2 receptor polypeptide, an IL-3 receptor
10 polypeptide, an IL-13 receptor polypeptide, an IL-4 receptor
11 polypeptide, a vascular endothelial growth factor (VEGF)
12 receptor, a granulocyte macrophage-colony stimulating factor
13 (GM-CSF) receptor polypeptide, an epidermal growth factor
14 (EGF) receptor polypeptide, an insulin receptor polypeptide,
15 an insulin-like growth factor receptor polypeptide,
16 transferrin receptor, estrogen receptor, a T cell receptor
17 (TCR) α -chain, a TCR β -chain, a CD4 polypeptide, a CD8
18 polypeptide, a CD7 polypeptide, a B cell immunoglobulin (Ig)
19 heavy chain, a B cell Ig light chain, a CD19 polypeptide, a
20 CD20 polypeptide, a CD22 polypeptide, a MAGE polypeptide, a

21 BAGE polypeptide, a GAGE polypeptide, a RAGE polypeptide, a
22 PRAME polypeptide, and a GnTV polypeptide.

1 9. The radiolabeled immunotoxin of claim 1, wherein the
2 radionuclide is selected from the group consisting of ^{90}Y ,
3 ^{186}Re , ^{188}Re , ^{64}Cu , ^{67}Cu , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{123}I , ^{125}I , ^{131}I , ^{211}At ,
4 ^{32}P , ^{177}Lu , ^{47}Sc , ^{105}Rh , ^{109}Pd , ^{153}Sm , ^{199}Au , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{124}I , ^{18}F ,
5 ^{11}C , ^{198}Au , ^{75}Br , ^{76}Br , ^{13}N , $^{34\text{m}}\text{Cl}$, ^{38}Cl , $^{52\text{m}}\text{Mn}$, ^{55}Co , ^{62}Cu ,
6 ^{68}Ga , ^{72}As , ^{76}As , ^{72}Se , ^{73}Se , and ^{75}Se .

1 10. A radiolabeled multimeric immunotoxin comprising:
2 (a) at least two monomers; and
3 (b) at least one radionuclide atom,
4 wherein each monomer comprises a targeting domain
5 and a toxic domain and is physically associated with the other
6 monomers,
7 wherein the targeting domain binds to a target
8 molecule on a target cell.

1 11. The radiolabeled multimeric immunotoxin of claim 10,
2 wherein each of said monomers further comprises one or more
3 coupling moieties and the physical association of the monomer
4 is by at least one of the one or more coupling moieties.

1 12. The radiolabeled multimeric immunotoxin of claim 11,
2 wherein the coupling moiety is a terminal moiety.

1 13. The radiolabeled multimeric immunotoxin of claim 12,
2 wherein the terminal moiety is a C-terminal moiety.

1 14. The radiolabeled multimeric immunotoxin of claim 11,
2 wherein the one or more coupling moieties are cysteine
3 residues.

1 15. The radiolabeled multimeric immunotoxin of claim 11,
2 wherein at least one of the one or more coupling moieties is a
3 heterologous coupling moiety.

1 16. The radiolabeled multimeric immunotoxin of claim 10,
2 wherein each of the monomers comprises the same amino acid
3 sequence.

1 17. An *in vitro* method of killing a target cell, the
2 method comprising culturing the target cell with the
3 radiolabeled immunotoxin of claim 1.

1 18. A method comprising:
2 (a) identifying a subject suspected of having a
3 pathogenic cell disease; and
4 (b) administering to the subject a radiolabeled
5 immunotoxin comprising a toxic domain, a targeting domain, and
6 at least one radionuclide atom, wherein the targeting domain
7 is a sFv antibody fragment that binds to a target molecule on
8 a target cell in the subject.

1 19. The method of claim 18, wherein the toxic domain is
2 a toxic polypeptide selected from the group consisting of: (a)
3 ricin, (b) *Pseudomonas* exotoxin (PE); (c) bryodin; (d)
4 gelonin; (e) α -sarcin; (f) aspergillin; (g) restrictocin; (h)
5 angiogenin; (i) saporin; (j) abrin; (k) pokeweed antiviral
6 protein (PAP); (l) a ribonuclease; (m) a pro-apoptotic
7 polypeptide, and (n) a functional fragment of any of (a)-(m).

1 20. The method of claim 18, wherein the toxic domain is
2 diphtheria toxin (DT) or a functional fragment thereof.

1 21. The method of claim 20, wherein the functional
2 fragment comprises amino acids 1-389 of DT.

1 22. The method of claim 18, wherein the target cell is a
2 cancer cell.

1 23. The method of claim 22, wherein the cancer cell is
2 selected from the group consisting of a neural tissue cancer
3 cell, a melanoma cell, a breast cancer cell, a lung cancer
4 cell, a gastrointestinal cancer cell, an ovarian cancer cell,
5 a testicular cancer cell, a lung cancer cell, a prostate
6 cancer cell, a cervical cancer cell, a bladder cancer cell, a
7 vaginal cancer cell, a liver cancer cell, a renal cancer cell,
8 a bone cancer cell, and a vascular tissue cancer cell.

1 24. The method of claim 22, wherein the target molecule
2 is Her-2/neu.

1 25. The method of claim 22, wherein the target molecule
2 is selected from the group consisting of a mucin molecule,
3 CEA, PSA, folate binding receptor, A33 alpha fetoprotein, CA-
4 125 glycoprotein, colon-specific antigen p, ferritin, p-
5 glycoprotein, G250, OA3, PEM glycoprotein, L6 antigen, 19-9,
6 P97, placental alkaline phosphatase, 7E11-C5, 17-1A, TAG-72,
7 40 kDa glycoprotein, URO-8, a tyrosinase, an interleukin-
8 (IL-)2 receptor polypeptide, an IL-3 receptor polypeptide, an
9 IL-13 receptor polypeptide, an IL-4 receptor polypeptide, a
10 VEGF receptor, a GM-CSF receptor polypeptide, an EGF receptor

11 polypeptide, an insulin receptor polypeptide, an insulin-like
12 growth factor receptor polypeptide, transferrin receptor,
13 estrogen receptor, a T cell receptor (TCR) α -chain, a TCR β -
14 chain, a CD4 polypeptide, a CD8 polypeptide, a CD7
15 polypeptide, a B cell Ig heavy chain, a B cell Ig light chain,
16 a CD19 polypeptide, a CD20 polypeptide, a CD22 polypeptide, a
17 MAGE polypeptide, a BAGE polypeptide, a GAGE polypeptide, a
18 RAGE polypeptide, a PRAME polypeptide, and a GnTV polypeptide.

1 26. The method of claim 18, wherein the method is a
2 method of killing a target cell in the subject.

1 27. The method of claim 26, wherein the radionuclide is
2 selected from the group consisting of ^{90}Y , ^{186}Re , ^{188}Re , ^{64}Cu ,
3 ^{67}Cu , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{123}I , ^{125}I , ^{131}I , ^{211}At , ^{32}P , ^{177}Lu , ^{47}Sc ,
4 ^{105}Rh , ^{109}Pd , ^{153}Sm , and ^{199}Au .

1 28. The method of claim 18, wherein the method is an
2 imaging method.

1 29. The method of claim 28, wherein the radionuclide is
2 selected from the group consisting of ^{186}Re , ^{188}Re , ^{64}Cu , ^{67}Cu ,
3 ^{212}Bi , ^{123}I , ^{131}I , ^{211}At , ^{177}Lu , ^{47}Sc , ^{105}Rh , ^{109}Pd , ^{153}Sm , ^{199}Au , $^{99\text{m}}\text{Tc}$,
4 ^{111}In , ^{124}I , ^{18}F , ^{11}C , ^{198}Au , ^{75}Br , ^{76}Br , ^{77}Br , ^{13}N , $^{34\text{m}}\text{Cl}$, ^{38}Cl , $^{52\text{m}}\text{Mn}$,
5 ^{55}Co , ^{62}Cu , ^{68}Ga , ^{72}As , ^{76}As , ^{72}Se , ^{73}Se , and ^{75}Se .

1 30. A method of making a radiolabeled immunotoxin, the
2 method comprising:

3 (a) providing a cell comprising a vector containing
4 a nucleic acid sequence encoding a protein, the nucleic acid
5 sequence being operably linked to a transcriptional regulatory
6 element (TRE);

7 (b) culturing the cell;
8 (c) extracting the protein from the culture; and
9 (d) attaching at least one radionuclide atom to the
10 protein,

11 wherein the protein comprises a toxic domain and a
12 targeting domain,

13 wherein the targeting domain is a sFv antibody
14 fragment that binds to a target molecule on a target cell,
15 wherein the target molecule is not a polypeptide of the CD3
16 complex.

1 31. A method of making a radiolabeled multimeric
2 immunotoxin, the method comprising:

3 (a) providing one or more cells, each of the cells
4 comprising a nucleic acid sequence encoding a monomer with a
5 different amino acid sequence, wherein the nucleic acid
6 sequence is operably linked to a TRE;

7 (b) separately culturing each of the one or more
8 cells;

9 (c) extracting the monomer from each of the
10 cultures;

11 (d) exposing the monomers to conditions which allow
12 multimerization of the monomers to form a multimer comprising
13 at least two monomers; and

14 (e) attaching at least one radionuclide atom to the
15 multimer,

16 wherein each monomer comprises a targeting domain
17 and a toxic domain,

18 wherein the targeting domain binds to a target
19 molecule on a target cell.

1 32. A method of making a radiolabeled immunotoxin, the
2 method comprising:

3 (a) providing a protein comprising a toxic domain
4 and a targeting domain; and
5 (b) attaching at least one radionuclide atom to the
6 protein,

7 wherein the targeting domain is a sFv antibody
8 fragment that binds to a target molecule on a target cell,
9 wherein the target molecule is not an ε chain of a T cell CD3
10 complex.

1 33. A method of making a radiolabeled multimeric
2 immunotoxin, the method comprising:

3 (a) providing a multimeric protein; and
4 (b) attaching at least one radionuclide atom to the
5 multimeric protein;

6 wherein the multimeric protein comprises at least
7 two monomers,

8 wherein each monomer comprises a targeting domain
9 and a toxic domain and is physically associated with the other
10 monomers,

11 wherein the targeting domain binds to a target
12 molecule on a target cell.

1 34. The radiolabeled multimeric immunotoxin of claim 10,
2 wherein the targeting domain is an antibody fragment.

1 35. The radiolabeled multimeric immunotoxin of claim 34,
2 wherein the antibody fragment is a sFv.

1 36. The radiolabeled multimeric immunotoxin of claim 34,
2 wherein the antibody fragment binds to a target molecule on a
3 T cell.

1 37. The radiolabeled multimeric immunotoxin of claim 34,
2 wherein the target molecule is a CD3 polypeptide.

1 38. The radiolabeled multimeric immunotoxin of claim 10,
2 wherein the targeting domain is a targeting polypeptide
3 selected from the group consisting of: (a) a cytokine; (b) a
4 ligand for a cell adhesion receptor; (c) a ligand for a signal
5 transduction receptor; (d) a hormone; (e) a molecule that
6 binds to a death domain family molecule; (f) an antigen; and
7 (g) a functional fragment of any of (a) - (f).

1 39. The radiolabeled immunotoxin of claim 1, further
2 comprising one or more additional targeting domains.

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